

for each allele of all of the eight alleles of DQA. The sequence included about 240 nucleotides of the intron I non-coding sequence and about 150 nucleotides (50 codons) of exon 2, the "polymorphic" exon of the gene. Polymorphisms in the coding region of several alleles that were unique to one allele were shown. Polymorphisms in the non-coding region of several alleles that were unique to one allele were also shown. Applicant's Attorney pointed out that it required very little skill to identify non-coding region polymorphisms characteristic of an allele because they were clearly apparent to any observer of the aligned sequences. In addition, Applicant's Attorney pointed out that, even with a highly polymorphic region such as the HLA alleles, unique polymorphisms were present for five of the eight alleles in fewer than 250 nucleotides of non-coding sequences. The Examiners agreed that it was within the level of skill to identify non-coding region sequence polymorphisms unique to an allele by looking at the aligned sequences. It is also clear that, for the DQA alleles, relatively short non-coding region sequences, clearly of a length that could be readily amplified as of the effective filing date of the application, contained informative polymorphisms that could be used to identify the alleles.

Declarations

At the interview, Applicant's Attorney discussed each of the declarations and how those declarations evidenced that informative polymorphisms similar to those for the DQA alleles in the exhibit shown at the interview are present throughout the eukaryotic genome. More specifically, Applicant's Attorney discussed Dr. Peter Gresshoff's Declaration and Examiner Sisson's concerns with the Declaration. In the Office Action, the Examiner stated:

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The crucial correlation between different soybean cultivars is discussed with intermediate results (microheterogeneity data conclusions) without any actual data for evaluation of this crucial element. A conclusion regarding specific soybean (sic, a specific soybean cultivar) is presented which is then generalized in an opinion that this supported a broad scope of enablement regarding loci for which the invention is applicable. There is an absence of even the simplest correlative data. Such absence of correlative data renders the declaration very weak as it is therefore based primarily on opinion. (page 3)

Applicant's Attorney pointed out that although the Declaration does not include data as noted by the Examiner, Dr. Gresshoff's Declaration contained numerous facts on which his opinion was based. Dr. Gresshoff also explained why the facts justified his conclusions. For example, Dr. Gresshoff described that he was sequencing a region of less than one kilobase of genomic DNA of specified soybean co-cultivars in what he believed to be an intergenic region near the supernodulation (NTS) locus. Dr. Gresshoff described why the NTS locus was considered a conserved locus. Dr. Gresshoff also described that the region contained informative polymorphisms which were indicative of the soybean co-cultivar being sequenced.

Dr. Gresshoff explained that he had no reason to believe the region was anything other than typical of intergenic regions of the soybean genome or of the genomes of other plants. Dr. Gresshoff also stated "the same correlation of non-coding region polymorphisms with coding region polymorphisms which is present in the HLA genes is also present in the soybean NTS gene." Dr. Gresshoff concluded that because the phenomenon of non-coding region polymorphisms indicative of coding region alleles was present in genes as diverse as the human HLA genes and the soybean NTS gene, that "the phenomenon is not limited to humans or even animals" and is present in eukaryotic genomes generally. Applicant's Attorney notes that the sequence variations found by Dr. Gresshoff were in an intergenic region, rather than in an intervening sequence. Thus, the informative non-coding region sequences are not

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limited to those associated with a genetic locus, such as the introns or flanking regions of the gene.

In summary, Dr. Gresshoff 's Declaration contained numerous facts and an explanation of how those facts supported his opinion. The Declaration is probative and, by itself, is sufficient evidence that the informative polymorphisms on which Dr. Simons' analysis method depends are present throughout the genome.

The Declaration of Dr. Leroy E. Hood was also discussed in the Office Action and at the interview. Dr. Hood is a noted expert in the field of genetics. In describing Dr. Hood's Declaration in the Office Action, the Examiner stated:

. . . Dr. Hood sequenced a "single" 100K base region in mouse and man and compared sequences. This comparison does not compare alleles in a multiallele (sic, multiallelic) locus but rather compares a single allele in mouse with a single allele in man. Dr. Hood admits that man and mouse diverged millions of years ago and concludes that the 70% homology over the non-coding region (95% of the compared sequence) under evolutionary pressure is striking and conclusive regarding the correlation between alleles and nearby non-coding regions. This conclusion is based on assuming that the divergence of mouse and man in evolution as to species is the same phenomenon as that which causes multiallelic variation.
(page 4)

Applicant's Attorney explained that the Declarations of Dr. Gresshoff and Dr. Rubinstein contained direct evidence of the presence of informative polymorphisms by virtue of describing the presence of unique polymorphisms in only one allele of a multiallelic locus. Dr. Hood's Declaration contains indirect evidence of the presence of such informative polymorphisms. More specifically, a significant degree of homology between alleles of a locus or between genes of a locus in different species is indirect evidence of the presence of informative polymorphisms, because the high degree of correlation between the sequences indicates the presence of non-random variation that provides such informative polymorphisms. Although Dr. Hood did not explicitly state this basic assumption, it is clear that his conclusion is based on

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the degree of homology found, between these vastly divergent species. In addition, Dr. Hood commented on both Dr. Simons' observation in HLA and Dr. Gresshoff's NTS facts in his Declaration. Therefore, Dr. Hood's Declaration is based not only on his sequencing information, but also on Dr. Simons' HLA data and the facts in Dr. Gresshoff's Declaration. Like Dr. Gresshoff's Declaration, Dr. Hood's Declaration is clear evidence of the presence of informative polymorphisms throughout the genome.

Applicant's Attorney also discussed Dr. Rubinstein's Declaration. In particular, Applicant's Attorney pointed out the portions of the publications described by Dr. Rubinstein indicating that the presence of informative polymorphisms characteristic of the allele of a multiallelic locus that were present in a sample. Each of the articles were briefly discussed, and relevant portions of the articles evidencing the presence of polymorphisms characteristic of alleles of a genetic locus were pointed out to the Examiners. As noted by Dr. Rubinstein in his Declaration, those informative polymorphisms were present in systems with limited polymorphism in humans as well as the human diversity genes and also in other mammals and even in insects. The range of species (human to drosophila) and level of conservation (HLA to highly conserved genes encoding vital enzymes) was pointed out. Therefore, it is believed that Dr. Rubinstein's Declaration is also clear evidence of the presence of informative polymorphisms throughout the genome.

In the Office Action, the Examiner described Dr. Hood's Declaration as follows:

. . . the scope of the instant invention lacks enablement when attempting to correlate coding and non-coding regions. (page 5)

This statement indicates that the enablement rejection was based, at least in part, on lack of evidence of operability of the claimed method. In other words, Applicant had failed to

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demonstrate that informative polymorphisms were generally present throughout the genome so that such informative polymorphisms could form the basis of the analysis. There is now overwhelming evidence of record that informative polymorphisms are present throughout the genome. Any aspect of the enablement rejection based on lack of evidence of operability for the full scope of the claimed method should now be moot. Removal of this basis of the rejection is respectfully requested.

The 35 U.S.C. § 112, First Paragraph Rejection

The section of the Office Action entitled "Rejection under 35 U.S.C. § 112, first paragraph" was also discussed at the interview. More specifically, the Examiner rejected Claims 1-13, 15, 16, 37, 39-43, and 48-50 under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner stated:

The specification does not set forth a repeatable procedure whereby one of skill in the art at the time the invention was made would be able to effectively determine where such relationships exist between exons and introns. Further, the specification does not enable one of skill in the art at the time the invention was made to perform such amplification reactions where the size of the nucleic acid to be amplified can be of virtually any length. (pages 7-8)

In addressing this aspect of the enablement rejection, Applicant's Attorney noted that the table presented at the interview showed a relatively small region of the sequence of DQA and demonstrated that identifying non-coding region polymorphisms characteristic of coding region polymorphism is a very simple matter, clearly below the level of skill in the art. In particular, it is clear that aligning multiple copies of different sequences of the same gene is a matter of routine. When those aligned sequences are grouped so that multiple copies of one allele are adjacent, one need only look for polymorphisms consistent within that group of sequences and not present in any of the other groups. Therefore, the

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identification of such informative polymorphisms was clearly shown to be well within the level of skill.

Furthermore, Applicant's Attorney pointed out that it was well within the level of skill at the filing date to detect the presence of such polymorphisms in a region of DNA by any one of a number of well-known techniques. Applicant's Attorney pointed out that an analysis of DQA in the specification was based on amplification of a relatively short region of intron 1 and 2 and all of exon 2. (The primers hybridize to sequences about 500 bp upstream from the 5' end of the second exon and 50 bp downstream from the second exon and produce amplified DNA sequences in the 700 to 800 bp range.) By amplifying a relatively short region of DNA (less than one kilobase) containing only about 550 non-coding region nucleotides, the resultant amplified DNA sequences unambiguously identified three of the eight alleles based on size differences of the amplified sequences on electrophoresis. By combining the amplification products with one of several different restriction endonucleases, all eight alleles and several haplotypes of the locus were identified. Applicant's Attorney noted that this identification did not even require knowledge of the particular non-coding region polymorphisms involved in the sequences to develop a typing system based on the informative non-coding region sequence polymorphisms. Only sufficient sequence information to identify conserved regions for primer sites was required.

Applicant's Attorney also pointed out that in the journal articles described by Dr. Rubinstein, one article sequenced particular regions and one used a combination of amplification and endonuclease digestion to identify alleles based on differences in non-coding region sequences. Examiner Sisson was clearly personally aware that amplification, endonuclease digestion, and electrophoresis were techniques that were

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readily practiced by those in skill at the time the application was filed.

Examiner Sisson questioned whether one of skill would be able to amplify all non-coding region sequences. In particular, the Examiner stated in the Office Action that some introns included more nucleotides than could be amplified. Applicant's Attorney pointed out that the application and declarations established that relatively short regions of DNA of a readily amplifiable size contained informative polymorphisms. Therefore, there was no question that sequences of an appropriate size to facilitate amplification contained informative polymorphisms. Applicant's Attorney pointed out that the claims do not require that a complete intron or any other non-coding region be amplified, only that non-coding region sequences be included in the amplified sequence. That the claimed method requires use of a region that is of an amplifiable size is inherent in the recited step of "amplifying genomic DNA" (Claim 1). Therefore, the claimed method clearly recites that a region of an amplifiable size is used. Since there is clear evidence that relatively short regions of non-coding sequences contain informative polymorphisms and that one of skill could readily identify and detect those informative polymorphisms, it is believed that this rejection has now been overcome. If the Examiner has any question, he is invited to telephone the undersigned attorney. Withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

The rejection of Claims 2, 6, and 50 under 35 U.S.C. § 112, second paragraph, for indefiniteness was also discussed. Claims 2 and 6 were said to be indefinite for use of the phrase "at least about". Claim 50 was rejected for use of the phrase "not more than about".

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In the Office Action, in describing the basis of the rejection of Claim 50, the Examiner stated:

Claim 50 is indefinite a (sic, as a) result of the phrase "not more than about one kilobase". The term "about" encompasses values above and below the indicated point. Therefore, the scope of "about" is in contradiction to the phrase "not more than".
(page 9)

It is well settled that the meanings of the term "about" and the phrase "not more than", or the similar phrase "at least" are definite. Why combining a well-understood term with a well-understood phrase is indefinite is unclear. In particular, values encompassed by the phrase "not more than about one kilobase" are "not more than" the largest value the term "about one kilobase" encompasses. Similarly, values encompassed by the phrase "at least about 300 nucleotides" are "at least" as large as the smallest value the term "about 300 nucleotides" encompasses. In other words, the value modified by the term "about" is given its normal interpretation. Then the phrase "not more than" or "at least" is given its normal interpretation in relation to the well-known interpretation of the number term. Thus, the meaning of the terms in the claims is clear and definite and would be understood by one of skill in the art. Withdrawal of the all the 35 U.S.C. § 112, second paragraph, rejections for indefiniteness is respectfully requested.

Rejection under 35 U.S.C. § 103

At the interview, the rejection of Claims 1-11 under 35 U.S.C. § 103 over Wai Kan et al. in view of Mullis was discussed. In particular, Applicant's Attorney pointed out that the use of non-coding region sequences for the types of analyses described by Kan et al. neither teach nor suggest the claimed invention. In particular, in the Office Action, the Examiner characterized Kan et al. as follows:

Wai Kan et al., (p. 5631 left col. second par.) teaches (sic, teaches) the identification of genetic polymorphisms at a restriction site which is close to

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the human β -globin structural gene. In the last sentence of said par., it is stated: "This type of polymorphism may be useful for linkage analysis, parental diagnosis, or anthropological studies."
(page 10)

As discussed at the interview, two articles, a review article by Graham et al. (*Blood*, 66:759-764 (1985)) and an article by Saiki et al. (*Science*, 230:1350-1354 (1985)), are of record in the present application and were used as part of the description of the teachings of the prior art in the parent case. (Copies of the articles were filed with the Information Disclosure Statement on September 23, 1992.) As described by Graham, restriction sites are sites of polymorphism that can be used in family studies to distinguish chromosomes to attempt to identify whether the child is affected by a disease. The site can only be used to determine which of the maternal chromosomes is inherited if the mother is heterozygous for the marker. Since the mother is a carrier, rather than affected by the disease, the mother necessarily has two different alleles of the disease gene and is therefore a heterozygote. If the mother can be homozygous for the site but have two different alleles of the gene, the site does not correlate with the alleles. In these studies, the site is used to distinguish between the mother's chromosomes. These sites clearly were not used to identify a particular allele of the disease gene since the first criteria for use of the site was that it was polymorphic in the heterozygous mother.

Although the focus of the Saiki article is on analysis of β -globin, the article describes DNA analysis methods used in prenatal diagnosis. The article states that both indirect and direct methods are used. Direct methods are said to be methods that do not require use of a family member and can be performed using only the DNA of the potentially affected child. The direct methods actually detect a coding region sequence polymorphism characteristic of the coding region allele. For example, the sickle cell disease is caused by a point mutation

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of the β -globin gene that alters a restriction site in exon 6 of the gene. Analyses that amplify a region around the site of the mutation and then cleave the site with the enzyme can be used to determine whether the site, and therefore the mutation, is present in the gene sequence. Another direct method is probing or amplifying genomic DNA to determine whether the sample contains an α -globin gene, the absence of the gene causing α -thalassemia. Use of cDNA probes (which bind to coding region sequences) is also disclosed.

The present invention relates to a direct analysis method in that the analyses do not require information regarding family members and can be performed using only the DNA to be analyzed. The claims do not prevent one from using any technique on a coding region sequence to detect a polymorphism in the coding region sequence, since the claims necessarily involve the use of polymorphisms in the non-coding region sequence to identify coding region polymorphisms. Therefore, those methods neither anticipate nor make obvious the present claims.

The other type of analysis described by Saiki is indirect. As stated by Saiki these analyses involve the use of DNA from family members to attempt to determine whether the child has inherited a disease-associated gene. As clearly discussed in the Graham and Saiki articles, the family studies are based on the presence of a marker which is a site of polymorphism. The polymorphic site is either within the genetic locus to be analyzed or sufficiently close to the locus to be analyzed so that the locus and the marker are likely to be inherited together with a high degree of probability. The first requirement for use of such markers in a particular test is that the maternal (or paternal) DNA is heterozygous for the marker. (See the Graham article at page 761, column 1, second full paragraph.) Therefore, clearly the "marker" cannot be used to directly determine which allele of the locus is

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present. This is evident since the unaffected parents are necessarily heterozygous for the disease gene and may or may not be heterozygous for the marker. That is, even if the marker is present in a non-coding region, the marker does not correlate with coding region alleles of the locus. As discussed at the interview, the claims do not encompass identifying a marker which could be used as a site of polymorphism to determine inheritance in family studies.

Applicant's analyses determine which allele is present in a sample, not which of two chromosomes was inherited. Such inheritance pattern studies were based on identification of a plurality of polymorphisms so that one or more of the polymorphisms would be heterozygous for each parent to facilitate determining which parental chromosome was transmitted. Such determinations did not identify polymorphisms characteristic of alleles or recognize that such characteristic polymorphisms were present in relatively short regions of non-coding region DNA. Therefore, the pending claims are patentable over the prior art genetic analyses described in the Graham and Saiki articles.

The Kan et al. article relates to use of a polymorphism for the types of indirect analysis methods described in the Graham and Saiki articles. As discussed at the interview, the quote from the Examiner clearly indicates that the site of polymorphism was not correlated to a particular allele of the β -globin gene. Rather, the authors identified a particular non-coding region polymorphism and speculated that the "polymorphism may be useful for linkage analysis, parental diagnosis, or anthropological studies."

Combining Mullis with Kan does not cure the deficiency, because Mullis does not teach or suggest that short non-coding region sequences contain informative polymorphisms. Removal of the § 103 rejection is respectfully requested.

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The Examiner indicated in the March 25, 1993 Office Action that the references submitted to the U.S. Patent and Trademark Office on December 21, 1992 "would not be printed on a patent should one be issued from the instant application; the title of these references was not included in said form". On September 14, 1994, Applicant's attorney submitted an Amendment and Form PTO-1449 that included the titles of the articles previously submitted. At that time, the titles of three of the articles were not submitted. The titles of those articles are provided on the attached Form PTO-1449.

All of the rejections in the Office Action having been overcome, it is believed that the application is now in condition for allowance. Early notice to that effect is respectfully requested. If a telephone conference would expedite the prosecution of this application, the Examiner is requested to telephone and confer with the undersigned Attorney.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class-mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C., 20231, on August 30, 1995.

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